PATHOLOGY AND ECOLOGY OF SPECIES OF RHIZOCTONIA RECOVERED FROM A REDUCED-TILLAGE EXPERIMENT MULTICROPPED TO RYE AND SOYBEAN IN FLORIDA

BY

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Abstract of Dissertation Presented to the Graduate Council
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Soil from a reduced-tillage experiment multicropped to 'Wrens Arbuzzi' rye and 'Bragg' soybean was sampled to a depth of 0-5 cm for *Rhizoctonia* spp. and other fungi. In the split-plot design, main plots were either subsoiled at a depth of 45 cm to break compacted subsurface layers or not subsoiled, and subplots were either tilled to a depth of 15 cm or not tilled. The soil was Arrendondo fine sand.

Nine anastomosis groups (AGs) or species of *Rhizoctonia* collected from the field were characterized. Six of these could be identified on the basis of cultural morphology. Isolates from two of these groups, *R. solani* AG 4 (anastomosis group of *R. solani*) and CAG 3 (binucleate anastomosis group of *Rhizoctonia* spp.), were pathogenic to soybean seedlings; only *R. solani* AG 4 isolates were pathogenic to rye seedlings.

Soil from the field was assayed approximately every 5 weeks for 820 days. Subsoiling had no significant influence ($p = 0.05$) on population densities

of any of the fungi tested, however, tillage and sample date were frequent and significant influences. Populations of Rhizoctonia spp., Pythium spp., and fungi from four other commonly isolated genera were often higher in no-till plots than in plots tilled to 15 cm. Population densities of R. solani AG 4 and Trichoderma spp. were significantly influenced by sample date, but not by tillage. Populations of R. solani AG 4 increased after either of the crops was planted in the field, and decreased as these crops matured.

Soil water potential was a significant influence on the survival and saprophytic activities of R. solani AG 4 in natural soil under laboratory conditions. In general, both of these activities were greatest in soils held at moderate water potentials (-2 to -15 bars).

Rhizoctonia solani AG 4 was not recovered from noncultivated Arrendondo fine sand in the vicinity of the experimental field but was frequently isolated from the same soil in which suscepts of the pathogen were planted. In this soil type, R. solani AG 4 is apparently restricted to areas in which the fungus may function as a parasite.

SECTION I

INTRODUCTION

Rhizoctonia solani Kühn is a plant pathogen capable of causing diverse symptoms on many hosts (5). This taxonomic species may be divided into several biological species or anastomosis groups (AGs) (46, 56, 77). Isolates within an AG anastomose with other isolates from the same AG but not with other isolates from different AGs. A total of five AGs of R. solani are found in the United States (26, 39, 46), two additional AGs are reported from Japan (34).

Isolates of AG 4 are common hypocotyl and seed pathogens of agronomic crops (4). They are destructive parasites of soybean causing seedling damping-off and hypocotyl rot (63). They have been reported infrequently as pathogens of monocots. In studies conducted on water agar, Murray (39) reported that an AG 4 isolate was capable of parasitizing barley seedlings. In field and greenhouse studies, Sterns and Jones (64) identified AG 4 pathogens of wheat causing sharp eyespot and Sumner and Bell (69) identified AG 4 isolates in Georgia as hypocotyl pathogens of corn. Rye has not been reported as a host for isolates from this AG.

Permeter (47) listed characters of R. solani distinguishing this fungus from other nonsporulating fungi. Among these criteria were possession of multinucleate vegetative cells, and, when formed, a teleomorphic stage of Thanatephorus cucumeris (Frank) Donk (71). Fungi closely resembling and sometimes mistaken for R. solani have been described (48). By grouping

isolates on the basis of hyphal anastomosis, Burpee et al (15) and Ogoshi et al (42, 43) recently characterized fungi similar to R. solani but possessing binucleate vegetative cells and producing a teleomorphic stage of Ceratobasidium spp

Binucleate isolates of Rhizoctonia spp. have been reported as plant pathogens (16, 36, 43, 78) Recently, Burpee et al. (14) demonstrated the pathogenicity of isolates Anastomosis Group 3 (CAG 3) and CAG 4 of Ceratobasidium spp. on several different hosts. They concluded that these fungi were potentially important soilborne pathogens. Still, little is known of the role or prevalence of binucleate species of Rhizoctonia in agronomic cropping systems.

Cropping systems utilizing reduced-tillage are gaining acceptance among farmers in the United States and elsewhere (49, 69). Reduced-tillage includes minimum-tillage and no-tillage which may be defined, respectively, as tillage essential and timely for producing the crop and as the planting of a crop in previously unprepared soil by opening the soil only enough for proper seed coverage (62) In 1982, reduced-tillage systems were used on 32% or 95 million acres of this country's cropland, and a total of 155 million acres of land managed with reduced-tillage is predicted by the year 2000 (55).

In the United States, reduced-tillage systems commonly are multicropped. Multicropping is defined as harvesting more than one crop per year from the same plot of land (62). Crop management systems combining reduced-tillage and multicropping are possible only in regions with a long growing season and adequate water supply. In such areas these combinations provide efficient ways to use land, equipment, and labor. When compared to conventional tillage, reduced-tillage results in an increase in soil retention of

water, plant nutrients and organic matter. Also, soil erosion due to wind or water is decreased by reduced-tillage management (49, 79).

Because many of these crop management systems have been proposed only recently, much research remains to be done on the feasibility of using these systems in a given region. One of the factors determining the feasibility of any cropping system is its performance with regard to plant pests. Plant pathologists have demonstrated that certain plant pathogens survive in crop residues (11, 13). Due to the increased crop debris found in soil managed under reduced-tillage, it might then be expected that these cropping systems will foster and possibly succumb to certain pathogens. This scenario has been suggested (69), and documented for certain pathosystems but not for others (12, 17, 69). If experience with pathosystems in different environments has demonstrated anything, however, it is that it is difficult to predict which pathogens may become a problem in a given cropping situation on the basis of one's intuition or past experience in related situations (6). Additional research on pathogens found in these nonconventional crop management systems and on factors influencing their occurrence in these systems is warranted.

During the past 40 years, ecological studies of R. solani have increased the understanding of the occurrence of this fungus in agronomic soils. Among the factors influencing its pathogenicity are temperature, presence of antagonistic fungi, and age, nutrition, size, and densities of inoculum in soil (7, 9, 18, 27, 29, 35, 40). Factors affecting the survival and saprophytic activity of R. solani have also been investigated; they include temperature, water potential, and nutrition of the fungus (18, 20, 44). The importance of soil moisture on the later activities has long been known (10, 23, 45). In much of

the work, however, ambiguous terms such as percent moisture holding capacity (45) or percent saturation (10) have been used to quantify the soil water status. In general, only in recent works have biologically meaningful terms for soil water status been used (9, 21, 67). Terms such as water potential (Ψ) or water activity (a_w=relative humidity) can be related to the availability of water for microbes (1, 25).

Rhizoctonia solani appears to survive best in dry soil. Benson and Baker (8) investigated the survival of R. solani AG 4 in soil incubated at water potentials of -0.7, -18, or -540 bars. At -0.7 or -18 bars, propagule densities increased 2-8 days after infestation, and then quickly decreased to a level maintained for the duration of the experiment (24 days). Propagule densities remained relatively constant at -540 bars for the entire experiment. Dubé (20) studied the survival of R. solani (probably AG 2) on agar culture medium and in soil adjusted to various water potentials. In some of these experiments, maximum survival of dormant hyphae in soil was recorded after 5 wk incubation at -940 bars; lower survival rates were recorded when hyphae were incubated in moister soils (-56 and -520 bars).

The growth of R. solani has also been studied under constant water potentials. Sterns and McCarver (67) demonstrated reduced radial growth in culture at -21.2 bars and no growth at -56.2 bars (AG not specified); their results agree with other work conducted on culture media by Dubé et al (21, AG 2 and AG 4) and Schneider (59, AG not specified), and in soil by Benson and Baker (8, AG 4). Also, in Dubé's (20) work, colonization of wheat straw by R. solani (probably AG 2) in soil occurred at -4.1 bars but not at water potentials of $\leq$-9.1 bars.

SECTION II

CHARACTRIZATION AND PATHOGENICITY OF SPECIES OF RHIZOCTONIA FROM A REDUCED-TILLAGE EXPERIMENT MULTICROPPED TO RYE AND SOYBEANS IN FLORIDA

Objectives

Reports have been made of AGs of R. solani occurring in a given field (4, 28); however, I know of no work characterizing both the multinucleate and binucleate constituents of a population of Rhizoctonia spp. in a field. An objective of this study was to identify and characterize species of Rhizoctonia found in a rye (Secale cereale L.) - soybean (Glycine max (L.) Merr.), multicropping, reduced-tillage experiment in Florida. Kuninaga et al. (33) have reported the occurrence of AGs of R. solani from noncultivated soils of Japan. Although AG 1, AG 2-1, AG 2-2, AG 5, AG 6, and AG BI were detected in this study, AG 4 was not found in these soils. In the present study, cultivated and noncultivated soils in the vicinity of the experiental field were assayed for the presence of multinucleate and binucleate species of Rhizoctonia. A final objective of this study was to determine the pathogenicity of species of Rhizoctonia recovered from the field to rye and soybeans. Portions of this work has been published previously (50, 53).

Material and Methods

Soil and plant samples described in this paper were obtained from an experimental field in Gainesville, Fl, in which 'Wrens Arbuzzi' rye was planted

in early November and harvested in late April and 'Bragg' soybean was planted in early May and harvested in October. The soil was Arredondo fine sand.

Isolations. Isolations of species of Rhizoctonia from soil were made on two selective media. Ko's (31) medium amended with 0.5 ppm benomyl (24) was used during initial portions of this study while Flowers' (68) medium was used thereafter. In comparative tests both media were rated effective in the selective recovery of Rhizoctonia spp. from soil (data not shown). Either medium was dispensed at approximately 12 ml per 9-cm Petri plate. In plates of media to be used for soil assays, 10 evenly spaced wells (1 cm in diameter) were made with a circular 1-cm die attached to a vacuum pump (Fig. 1).

Fifteen-gram subsamples were obtained from the field with a 2.5-cm soil core sampler; 35 to 45 subsamples were taken from each experimental plot and bulked for one combined sample. Each sample was assayed for the presence of species of Rhizoctonia within 18 hrs of recovery from the field. For each sample, 125 ml of dilute agar (0.25% Difco water agar) were mixed with the equivalent of 300 g of oven-dried soil in a Waring blender at low speed for 15 sec; 1 ml of the resulting suspension contained 1.0±0.1 g of soil. One ml of a suspension was pipetted into each of 10 plates of medium (0.1 ml per well) before incubation without light at 25°C. After 36-48 hr and 72 hr, plates were observed for fungal growth characteristic of species of Rhizoctonia. Identification of isolates as species of Rhizoctonia was confirmed by examination at 100X for the following characteristics: constriction of hyphae at branch points, occurrence of a septum in the branch near the point of origin, prominent septal pore apparatus, and absence of clamp connections. Hyphal tips of the isolates to be further characterized were transferred to Difco potato dextrose agar (PDA).

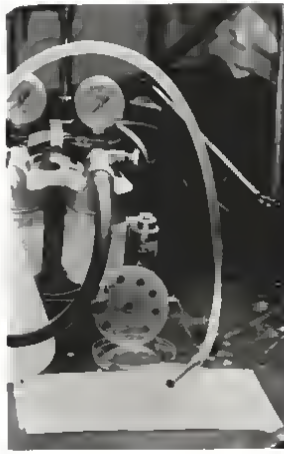

Fig. 1. Vacuum-pump apparatus for producing wells in media used for the selective recovery of Rhizoctonia spp from soil, and the arrangement of wells used.

Flowers' (68) medium or 1.5% water agar amended with 50 ppm streptomycin sulfate were used for isolation of Rhizoctonia spp. from rye or soybean tissue (seedling roots and stems) recovered from the field. Tissue was surface-disinfested with 0.25% NaOCl for 30 sec to 2 min (depending on size of tissue), rinsed with sterile, deionized water, and blotted dry with sterile paper towels before being placed on either medium and incubated at 25°C without light. Tissue plated on water agar plus streptomycin was observed after 18–24 hr incubation for growth of Rhizoctonia spp. while tissue plated on Flowers' (68) medium was observed after 24–48 hr.

A wet-sieving technique and a sieving-flotation technique (74) were used to isolate Rhizoctonia spp. from plant debris in soil taken from the field. 50 g samples of soil passed through a 2-mm sieve were used for each procedure. For wet sieving, soil was placed on an 850-μ sieve nested over a 500-μ sieve and gently washed under running tap water. Debris remaining on each sieve was dispersed individually onto water agar plus streptomycin in Petri plates. Sediment and debris separated by flotation from soil with the latter technique were recovered on filter paper by suction and removed by scraping with a spatula before placement on water agar plus streptomycin. Plates were incubated at 25°C without light and after 12–18 hr were observed for growth of Rhizoctonia spp.

Characterization of isolates. Isolates identified as species of Rhizoctonia were grown on PDA in 5-cm Petri plates at 25°C without light. A total of 269 isolates from soil, 11 from soybean hypocotyl tissue, 36 from rye coleoptile or stem tissue and eight from plant debris were examined (Table 2). Soil isolates were recovered between September and December, 1981 and soil debris isolates

were recovered in October of 1982. Isolates from soybean and rye were recovered from seedlings during 1981. After 3, 5, and 11 days of growth, isolates were classified on the basis of cultural characteristics (Table 1). Characteristics used for separation of isolates included pigmentation, density, and texture of mycelium; presence or absence of sclerotia; hyphal diameter, and radial growth rate.

At least three and as many as 64 isolates from each cultural type described in Table 1 were examined to determine the number of nuclei found in vegetative cells. Isolates were stained with a Giemsa-HCl procedure (76) or grown for 2 to 4 days on ICN agar (ICN Nutritional Biochemicals, Cleveland, OH 44128) and then stained with 0.5% aniline blue in lactophenol (14) or in glycerine (72). Examination of some isolates was repeated with 0.5% aniline blue in lactophenol.

The AG to which isolates in the different morphological types belonged was identified after anastomosis studies with tester isolates. All pairings were made with 0.5-cm plugs of mycelium taken from actively growing PDA cultures; isolates were opposed on slides covered with thin layers of 2.0% water agar. Slides were supported on glass rods over moist paper towels in 15-cm Petri plates, and incubated without light at 25°C. Regions of contact between opposing isolates were viewed at 100 or 400X for hyphal anastomosis (Fig. 2).

Preliminary studies (50) identified affinities of isolates from two of the seven cultural types with previously described anastomosis groups (15, 46). Isolates from type A anastomosed with R. solani AG 4 tester isolates (Isolates 458 and 770, Department of Plant Industry, Gainesville, FL, and isolate AG 4,

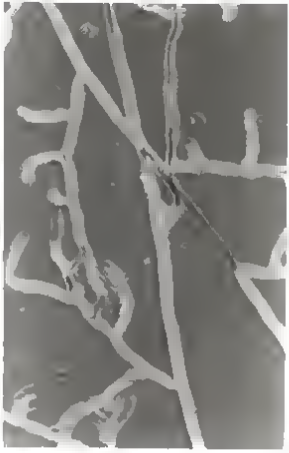

Fig. 2 Phase-contrast photomicrograph of region of contact between two opposing isolates of Rhizoctonia solani AG 4 showing extensive hyphal anastomosis. Note the killing reaction in cells on either side of contact points

Department of Plant Pathology, Univ Florida, Gainesville) and type B isolates anastomosed with a CAG 4 tester isolate (Burpee's Bn38 (15)). In later studies type C isolates anastomosed, although infrequently, with a CAG 3 tester isolate (Burpee's Bn31 (15)). Cultural type G was identified as R. zeae and was not included in anastomosis studies.

In the present study, a total of 160 isolates from cultural types A through F were paired with tester isolates from the experimental field. Tester isolates for cultural types A, B, and C anastomosed with tester isolates used in preliminary studies. In preliminary studies tester isolates used for cultural types D, E, and F anastomosed with isolates within the same cultural type, but not with any tester isolate obtained from Burpee (Bn1(CAG 1), Bn4(CAG 2), Bn31(CAG 3), Bn38 (CAG 4), and Bn37 (CAG 5) (15)) or Ogoshi (AH-1(AG-A), C-484(AG-Ba), C-348(AG-Bb), POER-2(AG-B), STC-5(AG-C), C-46(AG-D), OC-1(AG-E), SIR-1(AG-F), CY1-1(AG-G), STC-10(AG-H), and AV-2(AG-J) (43)) An additional tester isolate (FTCC 585 (CAG 7) (15)) from the Department of Plant Industry, Gainesville, Fl, did not fuse with tester isolates from the field. Also, 18 isolates from cultural type C were paired with the Bn31 tester isolate used in preliminary studies.

A soil-over-culture technique (73) was used to induce formation of basidia in isolates from the field, six isolates of R. solani AG 4, three isolates of CAG 3, and one isolate of CAG 4 were tested. Isolates were grown in Petri plates on PDA amended with 0.4% yeast-extract for 3 days or until colony margins reached the edge of the plate. Colonies were then covered with oven-dried soil and moistened as necessary (73). Three soils were used: Arredondo fine sand

from Florida at pH 6.0, Maury silty loam from Kentucky at pH 6.7, and Dothan loamy sand from Georgia at pH 6.0. In some experiments, soils were adjusted to pH 8.0 with 1M KOH. All soils were passed through a 2-mm sieve before use. Colonies covered with soil were then incubated under one of three light regimes: natural light (intensities to approximately 400 lux), fluorescent light (intensities to approximately 10 lux), and without light. Temperatures ranged from 21 to 27°C in the former two treatments and was held constant at 25°C in the later treatment. Each treatment had two replicates and experiments conducted with Arredondo fine sand were repeated. Cultures were observed for hymenia after 3, 8, and 20 days of incubation.

Two additional methods were tested for induction of basidia. Twenty-five isolates of R. solani AG 4 were tested using a technique devised by Adams and Butler (3). Some of these isolates were also grown on 1.5% water agar for 3 days. These cultures were incubated together in air-flow chambers under fluorescent light at 21 to 27°C. Maximum air-flow-rates of approximately 0.4 l/min were used for these studies.

Pathogenicity studies. Isolates of Rhizoctonia spp. recovered from soil, rye, or soybeans in the field were characterized as above. All isolates used anastomosed with tester isolates from the field. Isolates were grown on soybean stem tissue previously ground in a Wiley mill to pieces less than 3 mm in diameter. Five grams of tissue and 10 ml of deionized water were added to a 250-ml Erlenmeyer flask and autoclaved for 1 hr on each of two consecutive days. Tissue in each flask was then seeded with a 0.5-cm plug of a 5-to 7-day-old PDA culture of each of the isolates tested, and incubated for 4 wks at 25°C

without light. Soil was retrieved from the surface 10 cm of buffer plots in the field and passed through a 6-mm screen before use in pathogenicity experiments.

Three pathogenicity experiments were conducted with 'Bragg' soybean. For the first two experiments, inoculum for each isolate was recovered from a flask and mixed with sterile, deionized water in a Waring blender to yield an inoculum suspension. Blending fragmented mats of inoculum into small pieces (0.5 to 5.0 mm in diameter) that were easily mixed with treatment soils. Inoculum suspensions were added to autoclaved field soil (autoclaved for 1 hr on each of two consecutive days) and blended for 5 min in a Hobart mixer at low speed. Inoculum of certain isolates was also added to nonautoclaved soil and to autoclaved soil left to exposed to the air in a greenhouse for approximately 1 month (cured soil). Trichoderma harzianum Rifai aggr., T. hamatum (Bon.) Bain. aggr., Penicillium citrinum Thom, and Aspergillus ochraceus Wilhelm were the predominant fungi recovered from cured soil.

During the first experiment, 5 g of inoculum were mixed with 1 kg of each soil. The infested soil was then assayed immediately with Flowers' (69) medium for determination of inoculum densities. On the bases of these determinations, infested soil was mixed with noninfested soil to give a final calculated density of 100 propagules per 100 g of soil. At the end of this experiment, inoculum densities for these soils were again determined.

All tested isolates quickly and thoroughly colonized soil in the first experiment. At the end of the first experiment, inoculum densities reached 400 to 800 propagules per 100 g of soil. Therefore, in the second experiment, inoculum densities, which were initiated as in the first experiment, were

determined immediately prior to planting as well after the completion of the experiment. In this manner differences between inoculum densities calculated at planting time (100 propagules per 100 g of soil) and the actual inoculum densities in a given soil at this time were determined.

For the first two soybean experiments, seeds were surface-disinfested for 5 min in 0.25% NaOCl before planting to a depth of 3 cm in infested or noninfested (control) soil in 10-cm pots. Five seeds were planted in each pot and each treatment was replicated five times in a randomized complete block design on greenhouse benches. During both experiments temperatures ranged from 24 to 38°C and pots were watered daily. Soybean experiments 1 and 2 were terminated 16 and 13 days, respectively, after planting.

Only autoclaved soil was used during the third soybean experiment. Germinated seeds were planted 3 cm deep in each 10-cm pot before soil was infested with inoculum. Inoculum was manually broken into pieces approximately 1 mm in diameter. Approximately three pieces of inoculum of a given isolate were placed on the soil directly above each seedling before covering the soil with vermiculite. Control treatments with or without sterile soybean tissue were included. Pots were placed on a greenhouse bench and treatments were replicated four times in a randomized complete block design. During the experiment temperatures ranged from 20 to 32°C and pots were watered daily. This experiment was terminated 16 days after planting.

Only autoclaved soil was used during two experiments with 'Wrens Arbuzzi' rye. Five grams of inoculum of a given isolate were blended in 100 ml of sterile deionized water using a Brinkmann Polytron Homogenizer (Brinkmann, Westbury, NY 11590); propagules were 0.5 to 1.0 mm in diameter.

Two milliliters of a given inoculum suspension were then incorporated into 2 kg of soil by blending for 5 min in a Hobart mixer at low speed for a final inoculum soil dilution of 1:20,000 (W/W). For the first rye experiment, seeds were planted to a depth of 2.5 cm in soil in 10-cm pots; 10 seeds were planted per pot. Treatments were replicated five times and arranged in a randomized complete block design on a greenhouse bench. Temperatures during the experiment ranged from 20 to 26°C. Pots were watered daily and the experiment was terminated 18 days after planting.

For the second rye experiment, five 2-day-old seedlings were planted at a depth of 1 cm in 100 g of soil in a 100-ml plastic beaker perforated with three small holes to allow drainage. Three pieces of inoculum, approximately 1 mm in diameter, were placed on the soil surface directly above the seed before covering the soil with vermiculite. Treatments were replicated five times and randomized in a complete block design in an incubator with a diurnal light (12 hr light) and temperature (20°C days and 15°C nights) schedule. Plants were watered every third day and the experiment was terminated 18 days after planting.

At the end of all soybean and rye experiments, plants were washed under running tap water, surface-disinfested with 0.25% NaOCl, rinsed in sterile, deionized water, and blotted dry on sterile paper towels before placement on water agar plus streptomycin. Disease ratings for all plants were made the day experiments were completed. Disease severity was noted on soybean plants but rye seedlings were only examined for hypocotyl and root damage and discoloration, no attempt was made to rate the severity of disease found on these plants. Seedlings placed on water agar plus streptomycin were observed for growth of Rhizoctonia spp. after 24 hr incubation at 25°C without light

Histological examinations. Histological studies were conducted on 'Bragg' soybean and 'Wrens Arbuzzi' rye seedlings infected by AG 4 isolates. Plants grown and inoculated as for the third soybean and second rye experiment were examined beginning 3 days after inoculation. Surface infection structures were examined by gently washing seedlings free of soil and plant debris with tap water before staining for 30 sec to 2 min in 0.1% trypan blue in 10% aqueous acetic acid. When necessary tissue was destained in deionized water.

Occurrence of Rhizoctonia spp in areas outside the field. Noncultivated areas adjacent to the field and up to 500 m distant were assayed for Rhizoctonia spp using Flowers' (68) medium, several cultivated areas planted with suscepts of R. solani and in the vicinity (200 m to 30 km distant) were also assayed. All areas were sampled during the last 2 weeks in April, 1983, and at least two and as many as three sites were sampled within a given area.

Results

Isolates of Rhizoctonia spp recovered from the experimental field were assigned to one of seven cultural types based on cultural characteristics on PDA (Table 1). Isolates from cultural type A predominated, accounting for 39 to 91% of the total from any of the four sources of soil or plant tissue (Table 2), only isolates from cultural type A were recovered from all four sources. Isolates of types B through F combined to account for 13 to 40% of the total recovered for a source. Isolates from all seven cultural types were recovered from soil, isolates from five, three, and two of the types were recovered from rye tissue, soybean tissue, and soil debris, respectively.

Table 1. Cultural characteristics used to distinguish isolates of *Rhizoctonia* spp. grown on potato dextrose agar

Cultural[V] type	Species or anastomosis group	Characteristics used for classification	
		3 to 5 days growth	14 days growth
A	*Rhizoctonia*[W] *solani* AG 4	Mealy mycelial growth closely appressed to the medium surface, initial colony growth dense, spidery, and white in color; uneven colony borders, fast radial colony growth (15-21mm/day); hyphae 5-8u in diam (Types B-G had hyphae 3u in diam)	Non-rinded, chocolate brown sclerotia (2-5 mm diam); tan to chocolate brown colony pigmentation.
B	CAG 4[X]	Fine, mealy, mycelial growth in colony center becoming dense after 5-7 days growth; colonies usually form radial tufts of mycelium, colonies colored tan to brown; slow radial colony growth (approximately 10 mm/day)	Aerial mycelia with tan sclerotia (0.5-1.0mm diam); colonies colored cinnamon brown.
C	CAG 3[Y]	Some isolates form concentric growth rings; most isolates form buff colored clumps of moniliod cells closely appressed to the medium surface, buff-colored colonies; fast radial colony growth (12-18mm/day).	Most isolates eventually form tan to greyish brown sclerotia in the center and at the border of colonies (size variable)

Table 1. (Continued)

Cultural[v] type	Species or anastomosis group	Characteristics used for classification	
		3 to 5 days growth	14 days growth
D	This cultural[z] type is comprised of three binucleate anastomosis groups	Mycelial growth weakly to strongly aerial; some isolates form concentric growth rings, even colony borders; radiate, white to yellowish mycelial growth, thin hyphae (3u in diam).	Some isolates form aerial clumps of moniliaid cells; no brown pigmentation formed (colonies remain white to yellow in color).
E	Binucleate[z] group 1	Even colony margins, colonies are white; slow radial colony growth (8–10mm/day).	No sclerotia formed; colonies white and velvet-like in appearance; no sclerotia formed.
F	Binucleate[z] group 2	Fine, nonpatterned growth in colony center; radiate, brown-pigmented pattern visible from the underside of cultures.	Colonies dark brown.
G	Rhizoctonia zeae	Clamp connections lacking; white mycelial growth; white to salmon-colored sclerotial initials usually evident and embedded in medium.	Salmon-colored spherical sclerotia (approximately 1mm diam) embedded in the medium; colonies salmon-colored.

v Isolates from a reduced tillage experiment multicropped to rye and soybeans. Cultural types differentiated after growth on Difco potato dextrose agar at 25° C without light.

w Anastomosis group 4 of Parmeter et al. (45).

x Ceratobasidium anastomosis group 4 (15); isolates anastomosed with Burpee's Bn38 isolate.

y Ceratobasidium anastomosis group 3 (15), isolates infrequently anastomosed with Burpee's Bn31 isolate.

z Members of this group anastomosed with other isolates within the group but not with isolates from any previously described anastomosis group.

Table 2. Cultural types of isolates of Rhizoctonia spp. recovered from a field multicropped to wheat/soybean under reduced-tillage.

Cultural type	Species or anastomosis groups	Number of isolates per source			
		Soil	Debris	Soybean hypocotyl tissue	Rye coleoptile or stem tissue
A	R. solani AG 4	132 (49)[w]	6 (75)	10 (91)	14 (39)
B	CAG 4	4 (1)	0	0	0
C	CAG 3	37 (14)	0	1 (9)	1 (3)
D	BNG 3-5[x]	42 (16)	1 (13)	0	10 (28)
E	BNG 1[y]	14 (5)	0	0	4 (11)
F	BNG 2[y]	7 (3)	0	0	1 (3)
G	R. zeae	3 (1)	0	0	0
Other[z]		30 (11)	1 (13)	1 (9)	6 (17)
					36

[w] Percentage of total source within brackets.
[x] This cultural type was comprised of three anastomosis groups.
[y] Members of this group anastomose with other isolates in the group but not with isolates from any previously described anastomosis group.
[z] Isolates not fitting into any of the cultural types.

Nuclei of isolates were adequately stained for observation with the Giemsa-HCl and 0.5% aniline blue in lactophenol stains; poor resolution of nuclei was obtained with the 0.5% aniline blue in glycerine stain. Isolates from type A were multinucleate while isolates from types B through F were predominantly binucleate; three of 33 isolates of type D tested were trinucleate. Nuclei of isolates of type G (R. zeae) were not adequately stained with any of the stains used.

An AG 4 tester of R. solani from the field anastomosed with 93% of the isolates from cultural type A. Eighty-four percent of the isolates from type C anastomosed with a CAG 3 tester from the field; however, only 11% of these isolates fused with Burpee's CAG 3 tester (Table 3). The CAG 3 testers anastomosed with each other. Tester isolates for cultural types B, E, and F fused with isolates from their respective types 75, 66, and 75% of the time, while only 20% of the isolates from cultural type D anastomosed with the type D tester isolate from the same field (Table 3).

After 20 days of incubation, hymenia were not observed on any of the soil-over-culture plates. After 14 days, basidia were formed by three of the 25 isolates of R. solani AG 4 tested (Fig. 3) with the Adams and Butler (3) technique. One additional isolate of R. solani AG 4 formed basidia on 1.5% water agar. Basidium morphology indicated that these isolates were T. cucumeris (71).

Isolates of R. solani AG 4 were pathogenic to 'Bragg' soybean hypocotyls (Table 4), typical sunken, sharply defined lesions were caused by all isolates tested (Fig. 4A). Dome-shaped infection cushions were observed on plants inoculated with any of the three AG 4 isolates used in histological studies.

Table 3. Affinities of isolates of Rhizoctonia spp identified by cultural characteristics after 14 days growth on potato dextrose agar

Cultural type	Species/ anastomosis group of tester	Number of isolates per source[v]								
		Soil		Soybean hypocotyl tissue		Rye coleoptile or stem tissue		Total		Proportion verified (%)
		Tested	Verified[w]	Tested	Verified	Tested	Verified	Tested	Verified	
A	Rhizoctonia solani AG 4	32	29	5	5	8	8	45	42	93
B	CAG 6	6	4	2	2			8	6	75
I	CAG 3[x]	33	29	3	3	2	1	38	32	84
	CAG 3[y]	18	2	—	—			18	2	11
D	BNG 3 5[z]	35	7	—		10	2	45	9	20
I	BNG 1	11	7	—	—	4	3	15	10	66
I	BNG 2	3	2	1	1	—	—	4	3	75

[v] Isolates recovered from a reduced tillage experiment multicropped to rye and soybeans in Florida.
[w] Verifications for types A through F indicate anastomosis with a tester isolate from the field, except for type C where a tester isolate from outside he field was also used.
[x] A bridging isolate, I 6-6, was used as a tester
[y] Duspre's (15) Dn3I isolate was used as a tester isolate
[z] This cultural type is comprised of three anastomosis groups.

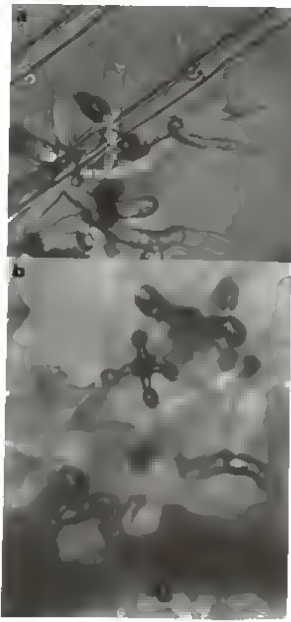

Fig. 3. Teleomorphic stage of an isolate of Rhizoctonia solani AG 4, Thanatephorus cucumeris, from the experimental field. a. Typical mature basidium with elongated sterigmata b Overhead view of arrangement of four basidiospores on a basidium.

(Fig. 5A). Root disease was not observed in soybeans exposed to AG 4 or any of the other isolates. All tested isolates of CAG 3 were also pathogenic to soybeans. All other isolates of Rhizoctonia spp. from the field were not pathogenic to 'Bragg' soybean. Apparent differences in virulence noted among AG 4 isolates during the first two experiments were possibly due to differences in inoculum densities and not due to differences in virulence among the various isolates. There was a positive correlation between final inoculum density and disease severity (r^2>0.770, Fig. 6)

Rhizoctonia spp. naturally present in the raw soil used in soybean pathogenicity tests made it impossible to rate the effect of individual isolates added to the soil. In the first two experiments, the severity ratings for disease caused by Rhizoctonia spp. in noninfested control treatments using raw soil were significantly greater than that found in noninfested control treatments using autoclaved soil (1.8 and 2.3 vs. 1.0 and 1.0, respectively; t-tests, $p = 0.05$). Also, Isolate X soil interactions were observed in studies using autoclaved soil and cured soil. Certain AG 4 isolates rapidly colonized both soils, and soybean seedlings became diseased when planted in them. However, other isolates capable of colonizing autoclaved soil were poor colonizers of cured soil (as determined by assay with Flowers' (6R) medium), and when soybeans were planted in cured soil infested with these isolates, typical disease symptoms were not observed or were greatly reduced. For these reasons only autoclaved soil was used in all pathogenicity experiments except the first two soybean experiments.

All tested AG 4 isolates were pathogenic to 'Wrens Arbuzzi' rye (Table 5). Symptoms and infection cushions resembling those described for other AG 4

Table 4. Pathogenicity of isolates of Rhizoctonia spp. to 'Bragg' soybeans

Description	Isolates: Source: Soil	Source: Soybean	Source: Rye	Total[t] tested	Total[u] pathogenic	Disease index[s]: Expt. 1[v]	Expt. 2[w]	Expt. 3[v]
R. solani AG 4	7	7	6	20	20	3.0±0.8[x] (1.9-4.0)	4.2±1.1 (2.6-5.0)	2.6±0.3 (2.2-3.0)
CAG 3[y]	9	0	0	9	9	2.6±0.7 (1.8-3.4)	1.7±0.5 (1.1-2.3)	1.3±0.3 (1.0-1.5)
CAG 4[y]	1	1	0	2	0	1.0	1.0	-
BNG 1[z]	2	0	0	2	0	1.0	1.0	-
BNG 2[z]	1	0	0	1	0	1.0	1.0	-
BNG 3[z]	2	0	0	2	0	1.0	1.0	-
BNG 4[z]	1	1	0	2	0	1.0	1.0	-

[s] Plants grown in autoclaved soil infested with a single isolate.
[t] Each isolate was tested in two of three experiments.
[u] Based on production of disease symptoms and recovery of isolate from plant(s) when experiment completed.
[v] Experiment terminated 16 days after inoculation.
[w] Experiment terminated 13 days after inoculation.
[x] Values are average mean rating for all isolates±standard deviation over the range of mean ratings for all isolates; Ratings 1 = healthy, 2 = lesion(s) covering to 1/4 circumference of hypocotyl, 3 = lesion(s) covering up to 1/2 circumference of hypocotyl, 4 = lesion(s) covering 3/4 circumference of hypocotyl, 5 = lesion(s) covering entire circumference of hypocotyl
[y] CAG = Ceratobasidium Anastomosis Group described by Burpee et al. (15).
[z] BNG = Binucleate Anastomosis Group from the experimental field and not previously described

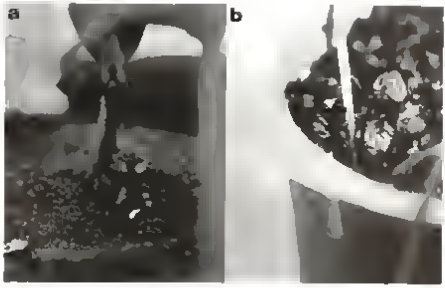

Fig. 4. Symptoms produced by an isolate of Rhizoctonia solani AG 4 from the experimental field. a. Hypocotyl and cotyledon infection of a 2 week-old 'Bragg' soybean seedling 10 days after inoculation. b. One-week-old Wrens Arbuzzi' rye seedling 5 days after inoculation. Arrow points to area on coleoptile dissolved by the pathogen revealing the stem. Although lightly colored, the stem is covered with several infection cushions.

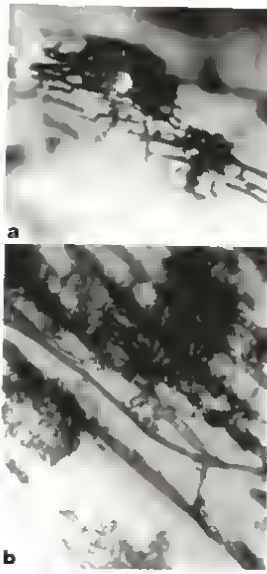

Fig. 5 Infection structures produced by an isolate of Rhizoctonia solani AG 4 from the experimental field. a. Dome-shaped infection cushion on a 10-day-old 'Bragg' soybean seedling 5 days after inoculation. b. Infection cushions on an 8-day-old 'Wrens Arbuzzi' rye seedling 7 days after inoculation. Structures were formed on stem after penetration of coleoptile.

Table 5. Pathogenicity of isolates of Rhizoctonia spp. to 'Wrens Abruzzi' rye.

Description	Isolates: Source			Total[u] tested	Total[v] pathogenic	Disease Index[t]	
	Soil	Soybean	Rye			Expt. 1[w]	Expt. 3[w]
R. solani AG 4	9	3	10	22	22	80(100-67)[x]	98(100-86)
CAG 3[y]	5	2	0	7	0	0	0
CAG 4[y]	0	1	0	1	0	0	0
BNG 1[z]	2	1	0	3	0	0	0
BNG 2[z]	1	0	0	1	0	0	0
BNG 3[z]	2	1	1	4	0	0	0
BNG 4[z]	3	0	0	3	0	0	0

t Plants grown in autoclaved soil infested with a single isolate.
u Most isolates were tested twice although seven AG 4 isolates were tested only once.
v Based on production of disease symptoms and recovery of isolate from plant(s) after the experiment was completed.
w Experiment was terminated 18 days after inoculation.
x Values are average mean rating for all isolates followed by the range of ratings for all isolates; Ratings = percentage of seedlings diseased.
y Ceratobasidium Anastomosis Group described by Burpee et al. (15)
z Binucleate Anastomosis Group from the experimental field that had not been previously described.

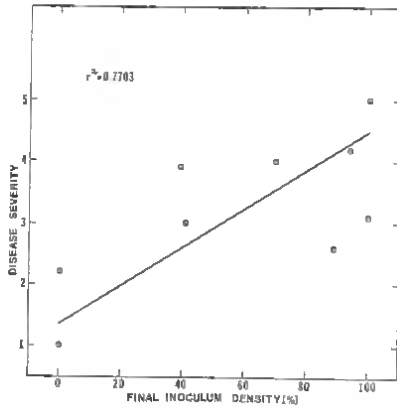

Fig. 6: Relationship of disease severity to final inoculum density in pathogenicity experiments with 'Bragg' soybean and isolates of Rhizoctonia solani AG 4

isolates on wheat (66) and barley (39) were observed on stems beginning 3 days after inoculation (Figs. 4b and 5b). If allowed to grow an additional 4 to 6 weeks, these infected plants either died or recovered to tiller and produce normal plants. All other isolates of Rhizoctonia spp. from the field were nonpathogenic on rye.

Isolates of Rhizoctonia spp. were not recovered from noncultivated soils in this study. However, isolates from cultural type A (AG 4) were commonly recovered from cultivated fields; isolates from cultural types C (CAG 3) and cultural type D were recovered less frequently (Table 6).

Table 6. Recovery of isolates of Rhizoctonia spp. from cultivated and noncultivated soil in the vicinity of a reduced-tillage experiment multicropped to rye and soybean

Area	Description of area sampled[v]		Cultural types recovered from area	Anastomosis group	Population[w] densities
	Noncultivated	Cultivated			
1	Bahia grass border of experimental field		None		
2	Bahia grass border of experimental field		None		
3	Bahia grass border of experimental field		None		
4	Bahia grass border of experimental field		None		
5	Woodlot (Carya sp., Zamia sp., and Quercus sp.)		None		
6	Fencerow (Rubus sp., Prunus sp., and Pinus sp.)		None A	AG 4[x]	5-19

Table 6. (Continued)

Area	Description of area sampled[v]		Cultural types recovered from area	Anastomosis group	Population[w] densities
	Noncultivated	Cultivated			
7		Minimum-tillage field, multi-	C	CAG 3[y]	0-1
		cropped to oats and soybean	D	BNG 3-5[z]	1-7
8		Clean-tilled field, multi-	A	AG 4	1-2
		cropped to rye and soybean	D	BNG 3-5	0-1
9		Minimum tillage field, multi-	A	AG 4	0-9
		cropped to rye and peanuts			
10		Clean-tilled sorghum field	None		
11		Minimum-tillage field, multi	None		
		cropped to rye and sorghum			
12		Clean-tilled tobacco field	A	AG 4	0-1
			D	BNG 3-5	1-4
13		Clean-tilled soybean field	A	AG 4	0-1
14		Clean-tilled rye field	A	AG 4	0-9
		(Planted to soybeans last yr)	D	BNG 3-5	1-15

[v] All soils in study were Arredondo fine sands.
[w] Isolations were made on a Flowers' (68) medium. Numbers proportion of 100 0.1 g aliquots of soil from wh[ich] Rhizoctonia spp. were recovered.
[x] Anastomosis Group 4 of Parmeter et al (46)
[y] Ceratobasidium Anastomosis Group 3 of Burpee et al (15).
[z] Binucleate Anastomosis Groups of Rhizoctonia not previously described.

SECTION III

POPULATION DYNAMICS OF PATHOGENIC AND NONPATHOGENIC FUNGI RECOVERED FROM A REDUCED-TILLAGE EXPERIMENT MULTICROPPED TO RYE AND SOYBEAN IN FLORIDA.

Objectives

Published work on the occurrence or population densities of pathogenic or nonpathogenic fungi in reduced-tillage and conventional-tillage soils have included data from one or two sample dates (19, 37, 70, 75). Although population density data from an expanded time-frame (> two sample dates) would probably increase the understanding of the ecology of these microorganisms in soils managed with these tillage practices, no published work contains data taken from more than two sample dates in succession. The purpose of the present study was to quantify populations of pathogenic and nonpathogenic fungi recovered from soil in a reduced-tillage experiment multicropped to rye (Secale cereale L.) and soybeans (Glycine max (L.) Merr.) in Florida over two cropping seasons for each crop. Portions of this work have been published previously (52).

Materials and Methods

For 4 years prior to the start of this study, plots in the field studied were not tilled and were either subsoiled at a depth of 45 cm to break compacted subsurface layers or not subsoiled. 'Bragg' soybean was planted in

May and harvested in October and 'Wrens Arbuzzi' rye was planted in November and harvested in April. At the beginning of this study, plots subsoiled and not subsoiled in the field were either tilled to a depth of 15 cm or not tilled, in the resultant split-plot design, subsoiled plots became main plots and tillage plots were subplots. Tillage and subsoiling treatments were imposed before the rye crop was planted each year and both crops were drill-planted. Treatments were replicated four times.

On 22 sample dates soil samples were taken from the surface 5 cm of treatment plots in the field; the sample dates spanned 820 days at intervals of approximately 5 wk. Approximately 40 subsamples were taken with a 2.5-cm-diameter soil core sampler from within plant rows in each treatment plot. Subsamples for each plot were pooled individually in plastic bags for transport to the laboratory; one pooled sample was taken from each subplot and 16 pooled samples were taken from the field on each sampling date. Bags containing soil were covered to inhibit moisture loss.

Pooled samples were assayed for fungi within 16 hr of recovery from the field. Percent soil moisture (g water/g oven-dried soil) was obtained for samples by weighing 5 to 10-g subsamples of pooled samples before and after drying at 100°C. For each pooled sample the equivalent of 300 g of oven-dried soil was suspended in 125 ml of 0.25% water agar by mixing in a Waring blender at low speed for 15 sec; 1 ml of these suspensions contained 1.0±0.1 g of soil. Soil suspensions were then used immediately in assays on selective agar media.

Ko's (31) medium amended with 0.5 ppm benomyl (24) was used for the isolation of Rhizoctonia spp. for the first six sample dates and Flowers' (68) medium was used for isolating Rhizoctonia spp. for the last 16 sample dates.

Soil suspensions were added to both media as described in Section 2. After 48 hr and 72 hr incubation at 25°C without light, media were observed for growth of Rhizoctonia spp., positive identifications were verified at 100 X under a compound microscope. Species of Rhizoctonia were identified as described in Section II. Incidence of Rhizoctonia species in soil was recorded as a proportion of 100 total wells (for each pooled sample) from which Rhizoctonia spp. grew; one positive well thus = 1%. Arc-sin transformations were performed on the data before analysis.

Difco cornmeal agar amended with 10 ppm pimaricin (Delvocid, Gist-Brocades N. V., Delft, Holland), 250 ppm ampicillin (Polycillin-N, Bristol, Syracuse, NY 13201), 10 ppm rifamycin SV (Rifampicin, Sigma Chemical Co., St. Louis, MO 63178) and 100 ppm PCNB (Terrachlor, 75% a.i., Olin Mathieson Chemical Corporation, Little Rock, AR 72203) was used to assay soil for Pythium spp. (30). Soil suspensions diluted 10 to 100 times with sterile 0.25% water agar were used; dilution rates were dependent on the time of year and the treatment being assayed. One milliliter of a dilution was applied to the surface of the solidified medium in a 9-cm Petri plate and spread evenly over the medium surface with the blunt end of an ethanol-disinfested test tube. Ten plates were used for each pooled sample. Plates were incubated at 25°C without light for 48 hr before examination for growth of Pythium spp. Pure cultures of isolates of Pythium spp. were identified to species by examining the isolates after growth on boiled grass blades placed in sterile pond water. Due to the consistent growth rate and colony morphology of isolates of P. irregulare Buisman on the selective medium, isolates of this species were routinely identified solely on these bases.

Difco potato dextrose agar amended with 1000 ppm Terginol NPX and 50 ppm chlortetracycline was used for estimating the population densities of common spore-forming fungi found in field soil. Depending on the time of year and treatment, 2,000 to 10,000-fold dilutions of soil suspensions made with sterile deionized water were used. Molten medium, cooled to 45-50°C, was added to soil dilutions in 9-cm Petri plates were agitated to disperse the soil dilution evenly throughout the medium. After the medium solidified, plates were incubated for 2-3 weeks at 25°C without light. Plates were examined then for fungal growth or after exposure to fluorescent light for an additional 1-3 days. These data and those for Pythium spp. were square-root tranformed before analysis. Data for all fungi were analyzed with a SAS (Statistical Analysis Systems, SAS Institute Inc., Cary, NC 27511) GLM (General Linear Models) program.

Weather data were obtained from a weather station on the grounds of the experimental farm on which the field was located. Missing temperature data were replaced by data from a weather station 30 km from the farm. Regression analyses with data for fungi and weather were performed with a SAS RSREG (Response Surface Regression) program.

Results

Fungi in the genera Penicillium, Aspergillus, Trichoderma, Fusarium, and Rhizopus accounted for 38 to 71% of all fungi recovered from a given treatment plot on a given sample date. Less frequently isolated fungi included species of the following genera: Laetisaria, Mortierella,

Myrothecium, Mucor, Neocosmospora, Neurospora, Paecilomyces, Phoma, Pyrenochaeta, Pythium, Rhizoctonia, and several others that were not identified. The following fungal species are listed in descending order of frequency of recovery for a given genus. Species of Penicillium recovered from the field included P. citrinum Thom, P. purpurogenum Stoll, and two other species that were not identified. Aspergillus ochraceus Wilhelm, A. clavatum Desm., A. flavus Link ex Gray, and A. niger van Tieghem constituted the total detectable Aspergillus population in field soil. Only two species of Trichoderma were routinely isolated during these studies: T. harzianum Rifai aggr. and T. hamatum (Bon.) Bain. aggr. Isolates of Fusarium and Rhizopus were not identified to species.

As described in Section II, nine anastomosis groups or species of Rhizoctonia were isolated from field soil; isolates of R. solani AG 4 and the binucleate Rhizoctonia spp. anastomosis group CAG 3 (15) were the most commonly isolated of these and were included in statistical analyses below. Pythium irregulare, P. acanthicum Drechsler, and several other unidentified species of Pythium were isolated from the field, only P. irregulare was included in statistical analyses.

In general, fungal population densities were influenced significantly by tillage and sample date; subsoiling effects were not significant ($p = 0.05$, Table 7). The effects of tillage and sample date on population densities of total fungi were highly significant, as was the tillage X sample date interaction; Rhizoctonia spp., Pythium spp., Penicillium spp., and Rhizopus spp. responded to these influences on variability in a similar manner. Tillage and sample date influenced significantly the population densities of P. irregulare, Aspergillus spp., and Fusarium spp.; tillage X sample date Table 7

Table 7. Effects of tillage, sample date, and subsoiling on population densities of fungi from soil recovered from a reduced-tillage system multicropped to rye and soybean in Florida.

Fungi	Source[U]	d.f.[V]	Mean-square	Probability of exceeding F-value
Total fungi	subsoiling(sub)[W]	1	0.7388	0.6122
	tillage (till)[X]	1	177.3067	0.0006*[Y]
	sub X till	1	0.1661	0.8492
	sample date (date)	19	39.1445	0.0001*
	date X sub	19	2.7531	0.4865
	date X till	19	7.2001	0.0006*
	date X sub X till	19	3.7279	0.1683
Rhizoctonia spp	sub	1	0.0141	0.3926
	till	1	0.4990	0.0008*
	sub X till	1	0.0019	0.7146
	date	20	0.2727	0.0001*
	date X sub	20	0.0163	0.0573
	date X till	20	0.0272	0.0001*
	date X sub X till	20	0.0105	0.3983
Rhizoctonia solani AG 4	sub	1	0.0030	0.7201
	till	1	0.0157	0.4169
	sub X till	1	0.0216	0.3455
	date	14	0.0541	0.0001*
	date X sub	14	0.0097	0.3147
	date X till	14	0.0134	0.0866
	date X sub X till	14	0.0080	0.5018
CAG 3[Z]	sub	1	0.0237	0.2426
	till	1	0.1068	0.0048*
	sub X till	1	0.0212	0.1001
	date	11	0.0092	0.0575
	date X sub	11	0.0086	0.0799
	date X till	11	0.0031	0.8248
	date X sub X till	11	0.0081	0.1105
Pythium spp.	sub	1	23.2819	0.1154
	till	1	35.3339	0.0477*
	sub X till	1	0.3017	0.7214
	date	19	47.6284	0.0001*
	date X sub	19	2.7927	0.6303
	date X till	19	7.8067	0.0012*
	date X sub X till	19	2.8496	0.6084

Table 7. (Continued)

Fungi	Source[U]	d f.[V]	Mean-square	Probability of exceeding F-value
Pythium irregulare	sub	1	20.1581	0.1798
	till	1	65.0666	0.0028*
	sub X till	1	1.2068	0.5316
	date	19	20.2470	0.0001*
	date X sub	19	2.7129	0.5494
	date X till	19	3.7788	0.1892
	date X sub X till	19	2.5618	0.8146
Penicillium spp.	sub	1	1.1970	0.0755
	till	1	60.6321	0.0070*
	sub X till	1	0.6726	0.6867
	date	19	20.5382	0.0001*
	date X sub	19	1.9854	0.4472
	date X till	19	4.3187	0.0036*
	date X sub X till	19	2.6149	0.1637
Trichoderma spp.	sub	1	14.6423	0.2304
	till	1	1.1280	0.6246
	sub X till	1	0.5325	0.7353
	date	19	3.9721	0.0007*
	date X sub	19	1.8744	0.2631
	date X till	19	2.1872	0.2631
	date X sub X till	19	0.9620	0.8945
Aspergillus spp.	sub	1	3.1525	0.4124
	till	1	35.1578	0.0138*
	sub X till	1	0.4313	0.7165
	date	19	18.6303	0.0001*
	date X sub	19	2.1080	0.0312*
	date X till	19	1.7411	0.1104
	date X sub X till	19	0.9824	0.6921
Fusarium spp.	sub	1	0.3317	0.2363
	till	1	6.3096	0.0209*
	sub X till	1	0.5165	0.4083
	date	19	24.7195	0.0001*
	date X sub	19	0.9812	0.7684
	date X till	19	1.8027	0.8967
	date X sub X till	19	0.7741	0.9123

Table 7. (Continued)

Fungi	Source[U]	d.f.[V]	Mean-square	Probability of exceeding F-value
Rhizopus spp.	sub	1	0.9043	0.3959
	till	1	20.9618	0.0017[*]
	sub X till	1	6.7148	0.0225[*]
	date	19	14.3348	0.0001[*]
	date X sub	19	0.8557	0.1750
	date X till	19	4.7090	0.0001[*]
	date X sub X till	19	0.9805	0.0546

[U] Source of variability.
[V] Degrees of freedom.
[W] Main plots in the split plot design were either subsoiled at a depth of 45 cm to break compacted subsurface layers of soil or were not subsoiled.
[X] Subplots in the split-plot design were either tilled to depth of 15 cm or not tilled.
[Y] * denotes significance at the 5% level.
[Z] Binucleate anastomosis group of Rhizoctonia (15).

interactions were not significant for these fungi. Population densities of R. solani AG 4 and Trichoderma spp. were affected significantly by sample date and densities of CAG 3 were affected significantly by tillage. Because subsoiling had no significant effect on fungal population densities, no-till and 15-cm till data were combined and analyzed separately for total fungi, Rhizoctonia spp., CAG 3, Pythium spp., and P. irregulare.

In no-till plots, mean population densities of total fungi were often statistically greater than those recovered from plots tilled to 15 cm ($p = 0.05$), especially during the rye crop after tillage treatments were imposed (Fig 7) Mean densities of Rhizoctonia spp. were also usually greater in no-till plots than in 15-cm till plots (Fig. 8). These differences were greatest following tillage and after statistically significant ($p = 0.05$). Within a given growth cycle of rye or soybean, mean densities of R. solani AG 4 were always greatest 18 to 45 days after planting (Fig. 9). These maximum densities decreased over the remainder of the crop's growth cycle and increased again after the next crop was planted. When t-tests were performed with data from the second year with mean densities from before or at planting and after planting these differences were significant ($p = 0.05$). Mean population densities of CAG 3 were higher in no-till plots than in plots tilled to 15 cm (Fig. 10). Due to high variability in these data, however, these differences were seldom significant ($p = 0.05$).

During the second year of the experiment, population densities of Pythium spp. in no-till plots were higher than those in 15-cm plots, although these differences were not often significant ($p = 0.05$); a similar pattern was not evident during the first year (Fig. 11). In comparisons of populations in 15-

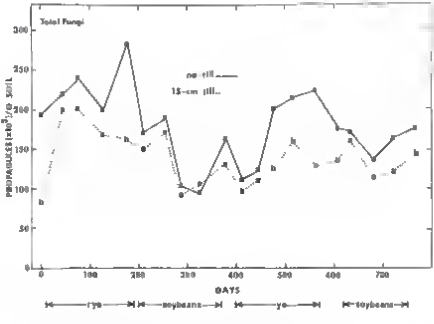

Fig. 7. Population densities of total fungi recovered from soil in a reduced tillage experiment in Florida multicropped to 'Wrens Arbuzzi' rye and 'Bragg' soybean. Rye was planted in November and was harvested in May, and soybeans were planted in May and harvested in October. At the beginning of this study, plots in the field were either tilled to a depth of 15 cm or not tilled prior to planting the rye crop each year. Points for a given date are not significantly different from each other if represented by the same letter ($p = 0.05$).

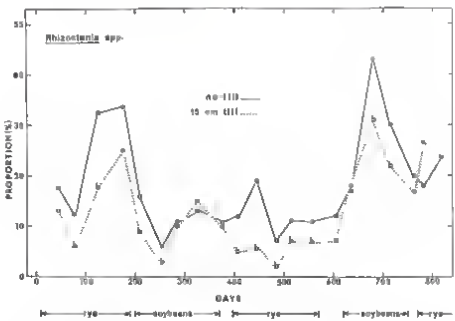

Fig. 8 Populat on densities of total species of Rhizoctonia recovered from soil in a reduced-tillage experiment in Florida multicropped to 'Wrens Arbuzzi' rye and 'Bragg' soybean. Rye was planted in November and was harvested in May, and soybeans were planted in May and harvested in October. At the beginning of this study, plots in the field were either tilled to a depth of 15 cm or not tilled prior to planting the rye crop each year. Proportions equal percentages of 0.1 g aliquots of soil placed on a selective medium from which species of Rhizoctonia were isolated. Points for a given date are not significantly different from each other if represented by the same letter ($p = 0.05$)

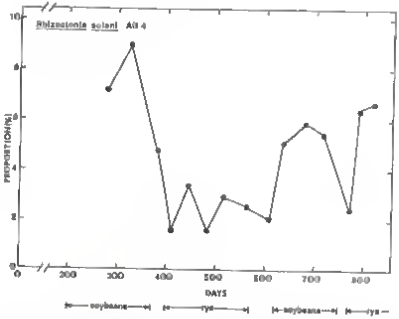

Fig 9. Population densities of Rhizoctonia solani AG 4 recovered from soil in a reduced-tillage experiment in Florida multicropped to 'Wrens Abruzzi' rye and 'Bragg' soybean. Rye was planted in November and was harvested in May, and soybeans were planted in May and harvested in October. At the beginning of this study, plots in the field were either tilled to a depth of 15 cm or not tilled prior to planting the rye crop each year. Proportions equal percentages of 0.1 g aliquots of soil placed on a selective medium from which R. solani AG 4 grew

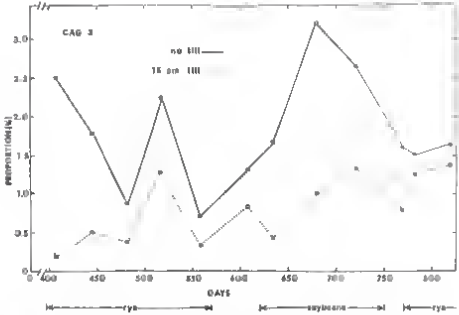

Fig. 10. Population densities of CAG 3(binucleate anastomosis group of Rhizoctonia) recovered from soil in a reduced-tillage experiment in Florida multicropped to 'Wrens Arbuzzi' rye and 'Bragg' soybean. Rye was planted in November and was harvested in May, and soybeans were planted in May and harvested in October. At the begining of this study, plots in the field were either tilled to a depth of 15 cm or not tilled prior to planting the rye crop each year. Proportions equal percenteges of 0.1 g aliquots of soil plated on a selective medium from which CAG 3 isolates were recovered. Points for a given date are not significantly different from each other if represented by the same letter ($p = 0.05$)

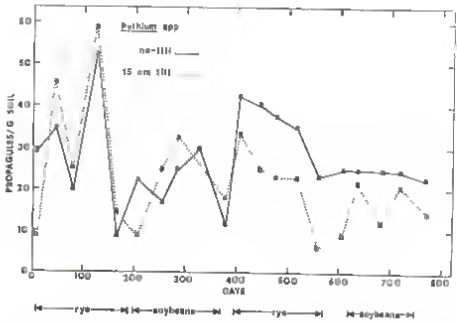

Fig. 11 Population densities of Pythium spp recovered from soil in a reduced-tillage experiment in Florida multicropped to 'Wrens Abruzzi' rye and 'Bragg' soybean. Rye was planted in November and was harvested in May, and soybeans were planted in May and harvested in October. At the begining of this study, plots in the field were either tilled to a depth of 15 cm or not tilled prior to planting the rye crop each year. Points for a given date are not significantly different from each other if represented by the same letter ($p = 0.05$).

cm till plots, increases in densities of Pythium spp. and P. irregulare were consistently noted for sample dates 7 to 55 days after planting versus those at or shortly before planting. These changes in population densities were always significant for Pythium spp., but were significant for P. irregulare only at the beginning of both rye crops (t-tests, p = 0.05; data not shown). The same trend was noted for densities of Pythium spp. and P. irregulare in no-till plots only during the second rye crop. Densities of P. irregulare in no-till plots were always higher than those in 15-cm till plots during the second year of the experiment, but not consistently so during the first year. During the second year, these differences were often significant (p = 0.05, Fig. 12).

Regression models for population densities of Rhizoctonia spp., R. solani AG 4, CAG 3, Pythium spp., and P. irregulare were built using terms incorporating sample date, soil moisture, and temperature. Fifty-seven percent of the variability found in data for Rhizoctonia spp. was accounted for in a model using linear and quadratic combinations of the above factors (Table 8); cubic terms were not significant (p = 0.05) and were not used in the model. The same model accounted for only 23% of the variability in data for R. solani AG 4; only linear terms in the model were significant (p = 0.05, Table 8). R-square values of 0.11 to 0.33 were obtained with models for CAG 3, Pythium spp., and P. irregulare incorporating linear, and quadratic terms (data not shown). Regression models were also built including a term for population densities of Trichoderma spp. with the above weather terms; densities of Trichoderma spp. was not a significant term in any of the models.

Table 6 Influence of sample date, soil moisture, and temperature on population densities of Rhizoctonia spp. and R. solani AG 4 in soil from a reduced-tillage experiment multicropped to rye and soybean in Florida.

Variable	Regression	d.f.[w]	sum of squares	r^2	F-ratio	pr>[x] F-value
Rhizoctonia spp.	linear	3	2.7970	0.4442	60.56	0.0001*[y]
	quadratic	3	0.3975	0.0631	8.61	0.0001*
	cross-product	3	0.4228	0.0672	9.15	0.0001*
	total regress	9	3.6173	0.5745	26.11	0.0001*

Parameter	d.f.	t-ratio	pr > |t-value|[z]
sample date (date)	1	-3.06	0.0026*
soil moisture (soil)	1	-2.11	0.0366*
temperature (temp)	1	-1.82	0.0710
date X date	1	1.27	0.2051
date X soil	1	2.58	0.0108*
soil X soil	1	2.87	0.0047*
date X temp	1	4.37	0.0001*
soil X temp	1	-2.44	0.0158*
temp X temp	1	-1.32	0.1857

Table 8. (Continued)

Variable	Regression	d.f.[w]	sum of squares	r^2	F-ratio	pr>[x] F-value
R. solani AG 4	linear	3	0.4070	0.2002	15.11	0.0001*
	quadratic	3	0.0017	0.0008	0.06	0.9738
	cross-product	3	0.0628	0.0309	2.33	0.0746
	total regress	9	0.4715	0.2318	5.84	0.0001*

Parameter	d.f.	t-ratio	pr > | t-value |
date	1	-0.98	0.3283
soil	1	-0.26	0.7980
temp	1	-0.10	0.9194
date X date	1	-0.45	0.6518
date X soil	1	2.46	0.0149*
soil X soil	1	-0.66	0.5076
date X temp	1	2.60	0.0111*
soil X temp	1	-2.24	0.0264*
temp X temp	1	-1.50	0.1348

[w] Degrees of freedom.
[x] Probability of exceeding the F-value.
[y] Denotes significance at the 5% level.
[z] Probability of exceeding the absolute value of t.

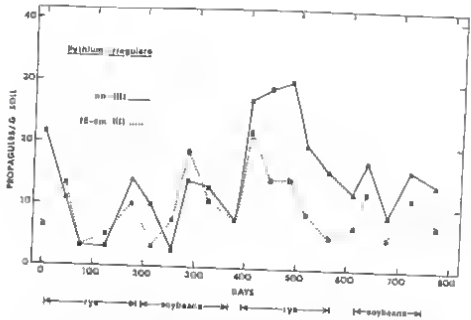

Fig. 12 Population densities of Pythium irregulare recovered from soil in a reduced-tillage experiment in Florida multicropped to 'Wrens Arbuzzi' rye and 'Bragg' soybean. Rye was planted in November and was harvested in May, and soybeans were planted in May and harvested in October. At the beginning of this study, plots in the field were either tilled to a depth of 15 cm or not tilled prior to planting the rye crop each year. Points for a given date are not significantly different from each other if represented by the same letter ($p = 0.05$)

SECTION IV

INFLUENCE OF WATER POTENTIAL ON THE SURVIVAL AND SAPROPHYTIC ACTIVITY OF RHIZOCTONIA SOLANI AG 4 IN NATURAL SOIL.

Objectives

The objectives of the present study were to determine the influence of soil moisture on the survival and saprophytic activity of R. solani AG 4 in natural soil under controlled environmental conditions. Portions of this work have been published previously (51).

Materials and Methods

All survival and saprophytic colonization studies were conducted with an Arredonda fine sand from the experimental field described in Section II. Soil was passed through a 2-mm sieve before use. All isolates of R. solani AG 4 tested were recovered from the field and pathogenic to 'Wrens Arbuzzi' rye and Bragg' soybean in tests described in Section 2. Isolates were grown on Difco potato dextrose agar for 3-5 days at 25°C without light. A 5-mm plug from each of these cultures was then used individually to infest mature soybean stem tissue from the field. The tissue was previously ground in a Wiley mill to a particle size of $\leq$ 3 mm in diameter and autoclaved for 1 hr on

each of two consecutive days. These cultures were incubated for 4 wk at 25°C without light before use. This inoculum was then air-dried for 4 hr before passage through 850-μ and 500-μ sieves; inoculum remaining on the 500-μ sieve was used for all experiments. Based upon germination on 1.5% Difco water agar, viability of pieces of inoculum used in all experiments ranged from 85 to 95%.

Survival studies. Survival of isolates of R. solani AG 4 was tested in the laboratory in natural soil held at constant water potentials. Buchner funnels, 10 cm in diameter and fitted with fritted glass disks, were suspended over water columns to achieve matric potentials of 0, -0.05, and -0.2 bar as described by Dunway (22). Two hundred grams of soil were then placed in each funnel and brought to saturation. Soil was tamped to achieve a bulk density of approximately 1.5 g/cc. Water columns were then lowered predetermined distances to achieve one of the three matric potentials. Funnels were covered with Parafilm (American Can Co., Greenwhich, CT 06830) fastened with rubber bands and aluminum foil. Soil in funnels equilibrated overnight before use in an experiment. Temperatures in the laboratory ranged from 23 to 28°C during any of these experiments.

Two additional water potentials were achieved by supporting 50 g lots of soil in 9-cm Pyrex Petri plates over NaCl solutions at -2 or -10 bars (57). These water potential systems were enclosed in desiccators covered with Parafilm, to allow gas diffusion, and sealed with stopcock grease. Soils were allowed to equilibrate 4 days before use. In a preliminary test, air-dried soil incubated in chambers prepared to adjust soil to -2 bars failed to take up additional water after 4 days. These water potential systems were placed in an incubator without light at 25±0.5°C.

Each funnel or Petri plate containing soil in the above systems was considered an experimental unit. Some of the experimental units were not infested and were used as control treatments. A localized arrangement of inoculum of four isolates was tested. In the remaining experimental units, approximately five pieces of inoculum were placed in soil at each point on a 5 X 5 grid; the 25 points on the grid were separated from one another by 1.5 cm in a uniform fashion (Fig. 13). At each of five time intervals after infestation (0, 4, 10, 25, and 55 days), five soil samples were taken with a 4-mm cork borer from each experimental unit at points on the grid. Sample sites on the grid were chosen randomly but were consistent for all treatments on a given sample date. Soil samples were assayed on Flowers' (68) medium for the presence of R. solani as described in Section 2. Treatments were replicated over time in three experiments in a split-plot design, two of each combination of isolate X water potential (experimental unit) were used in each experiment and were considered main plots; sample dates were considered subplots. Data in Fig. 14 are combined results for the recovery of Isolate I-19 over time in three experiments. These and all remaining data in this paper were arc-sin transformed and analyzed with SAS (Statistical Analysis Systems, SAS Institute Inc., Cary, NC 27511) GLM (General Linear Models) and RSREG (Response Surface Regression) programs.

In other survival studies, inoculum was dispersed in soil. Inoculum of one of the isolates (I-19) of R. solani AG 4 tested above was used to infest air-dried soil (water potential of approximately -1500 bars estimated after soil equilibrated in air at 0 relative humidity of 32%). One-half of a gram of inoculum was added to each kg of soil and blended in a Hobart mixer at low speed for 3 min. While blending, water was added to some of the soil lots to

Fig. 13. Buchner funnel used in survival studies, plastic template used to infest soil at the beginning of each experiment, and cork borer used to sample soil.

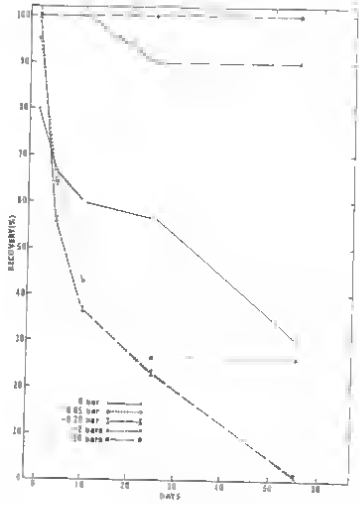

Fig. 14. Survival of inoculum of Rhizoctonia solani AG 4 (Isolate I 19) in natural soil held at constant water potentials. Each point equals the percentage of a combined total of 30 localized sources of inoculum in three experiments from which R. solani AG 4 was isolated.

achieve water potentials of approximately -100, -15, -8, -3.5, -0.1, and -0.05 bars; water potentials for the first four treatment soils were determined by thermocouple psychrometry, and water potentials for the latter two treatment soils were determined from a water characteristic curve for the treatment soil (Fig. 15). This curve represents desaturation data from an experiment in Buchner funnels. Nonunfested soils for each of the water potentials were used as control treatments.

The equivalent of 100 g of oven-dried soil was added to each of 15 100-ml tripour beakers, tamped to obtain bulk densities of approximately 1.5 g/cc, and quickly covered with Parafilm to inhibit moisture loss. Soil in beakers was incubated at 25°C without light. Soils were assayed for R. solani AG 4 with Flowers' (68) medium the day of infestation. Soils were also sampled 3, 6, 9, and 12 days after infestation by combining soil from three beakers for each treatment on each sample date for assay with the selective medium. This experiment was conducted twice and portions of it were repeated a third time. Data in Figs. 16 and 17 for all water potentials but -1500 bars are combined results for two experiments; data for -1500 bars are from one experiment. Regression data in Table 10 are from one experiment with a split-plot design; water potential treatments were main plots and sample dates were considered subplots. Each treatment was replicated three times.

Saprophytic colonization studies. The influence of soil water potential on the saprophytic activity of R. solani AG 4 in natural soil was tested. Inoculum of Isolate I-19 was prepared as above and used to infest soil held at each of six water potentials (-1500, -15, -8, -3.5, -0.1, and -0.05 bars); 0.5 g of inoculum (250 to 500 μ in diameter) was added to each kg of soil. Soils were moistened

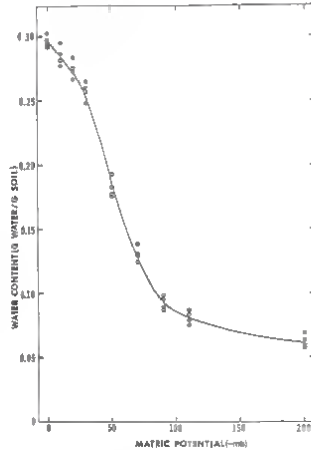

Fig. 15. Water characteristic curve for Arredondo fine sand used in survival and saprophytic colonization studies.

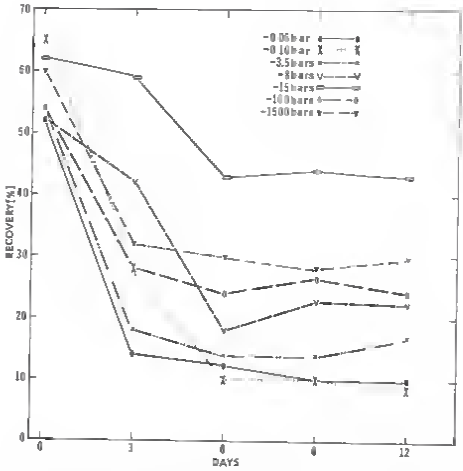

Fig. 16 Survival of inoculum of Rhizoctonia solani AG 4 (isolates 1–19) in a dispersed arrangement in natural soil held at constant water potentials. Each point equals a proportion of a total of 100 0.1 g aliquots of soil in two experiments from which R. solani AG 4 was recovered.

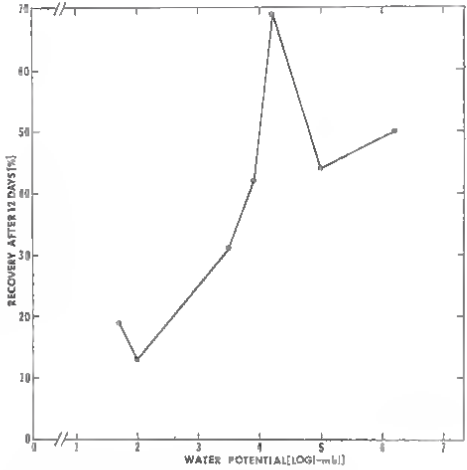

Fig. 17 Survival of inoculum of Rhizoctonia solani AG 4 (isolate 1-19) in a dispersed arrangement in natural soil held at constant water potentials. Each point equals a proportion of a total of 100 0.1 g aliquots of soil, after 12 days incubation, from which R. solani AG 4 was recovered (combined data from two experiments.)

as above and added to 100-ml plastic tripour beakers. Control treatments were not infested. Ten 1-cm long segments of mature Wrens Arbuzzi rye stems were placed in soil in each of the beakers before tamping the soil to achieve a bulk density of approximately 1.5g/cc (taking into account the volume of soil displaced by the stem pieces). Beakers were then covered quickly with Parafilm before incubation at 25°C without light. Stem sections were assayed for the presence of R. solani AG 4 prior to exposure to infested soil, and 1, 2, 3, 5, and 12 days after exposure; 30 stem pieces were assayed for each treatment on each sample date. The experiment was repeated once.

Results

In regression analyses with survival data, there was no significant isolate X water potential interaction; general trends evident for isolate 1-19 at each water potential are also valid for the 1-5, 1-13-1, and 2-11-19 isolates used in these experiments. When survival of the four isolates was compared over all five water potentials, however, one isolate, 1-13-1, survived significantly better than the remaining three (Table 9). Also, based on linear, quadratic, and cubic regression, survival of all isolates at -0.05 and -0.2 bar was significantly lower than that at -10 bars. Regression lines for 0 and -2 bars were not significantly different ($p = 0.05$) than that for -10 bars.

In survival tests with a dispersed arrangement of inoculum, survival of R. solani AG 4 (isolate 1-19) at -15 bars was significantly greater than that at -0.05 bar (Figs. 16 and 17). Linear, quadratic, and cubic regression lines for all other water potentials tested were not significantly different from that for -0.05 bars (Table 10).

Table 9. Influence of isolate, water potential, and sample date on survival of Rhizoctonia solani AG 4 in natural soil

Source[p]	d.f.[q]	sum of squares	mean square	F- value	pr>F-value[r]	r^2
Model	22	18.1392	0.8245	32.9044	0.0001	0.7441
Error	249	6.2394	0.0251			
Corrected total	271	24.3786				

Source	d.f	sum of squares	F-value
Isolate (Isol)[s]	3	1.1596	0.0001
Water potential (Ψ)[t]	4	9.3407	0.0001
Sample date (date)[u]	1	9.9769	0.0001
Date X date	1	0.8230	0.0001
Date X date X date	1	0.3533	0.0002
Date X Ψ	4	1.2903	0.0001
Date X date X Ψ	4	0.8971	0.0001
Date X date X date X Ψ	4	0.2982	0.0200

Parameter[v]	Class levels[w]	estimate[x]	t for H_0:[y] parameter = 0	pr>[z] | t-value |
Intercept		1.0585	21.60	0.0001
Isolate				
	1-19	-0.1125	-4.21	0.0001
	1-5	-0.1084	-3.88	0.0001
	2-11-19	-0.1154	-4.42	0.0001
	1-13-1	0.0000	.	.
Ψ	0 bar	-0.1419	-2.20	0.0284
	0.05 bar	0.0008	0.01	0.9906
	-0.2 bar	-0.0628	-0.96	0.3391
	-2 bars	0.0271	0.41	0.6816
	-10 bars	0.0000	.	.

Table 9 (Continued)

Parameter[v]	Class levels[w]	estimate[x]	t for H_0:[y] parameter = 0	pr>[z] |t-value|
Date		0 0006	0.05	0 9635
Date X date		0 0000	0.01	0 9906
Date X date X date		0.0000	-0.05	0.9586
date X Ψ	0 bar	-0 0279	-1.56	0 1200
	-0.05 bar	-0 0758	-4.33	0.0001
	-0.2 bar	-0 0699	-3.87	0 0001
	-2 bars	-0 0071	-0.39	0.6990
	-10 bars	0 0000	.	.
Date X date X Ψ	0 bar	0.0006	0 64	0.5208
	0.05 bar	0.0029	3 04	0.0026
	-0 2 bar	0.0026	2.65	0.0085
	-2 bars	0.0003	0.31	0.7551
	-10 bars	0.0000	.	.
Date X date X date X Ψ	0 bar	0.0000	-0.39	0.6947
	-0.05 bar	0 0000	-2.57	0 0109
	-0.2 bar	0.0000	-2.28	0 0232
	-2 bars	0 0000	-0.35	0.7251
	-10 bars	0 0000	.	.

[p] Source of variability.
[q] Degrees of freedom
[r] Probability of exceeding the value of F.
[s] Isolates of R. solani AG 4 used in survival studies.
[t] Water potentials at which soil was incubated in these studies.
[u] Sample dates on which soil was assayed for survival of R. solani AG 4.
[v] Parameters included in the regression model.
[w] Class levels for which regressions were conducted.
[x] Intercept and coefficient estimates for parameters in the model.
[y] Students t-test value for the null hypothesis that the parameter equals zero.
[z] Probability of exceeding the absolute value of t

Table 10. Influence of water potential and sample date on survival of Rhizoctonia solani AG 4 in natural soil.

Source[U]	d f.[T]	sum of squares	mean square	F- value	pr > F-value[S]	r^2
Model	23	803.0019	24.9131	16.04	0.0001	0.4640
Error	426	927.4981	2.1772			
Corrected total	449	1730.5000				

Source	d-f	sum of squares	F-value	pr > F value
Water potential (Ψ)[T]	5	311.8067	28.64	0.0001
Sample date (date)[V]	1	328.8178	151.03	0.0001
Date X date	1	95.0127	43.64	0.0001
Date X date X date	1	3.6100	1.66	0.1986
Date X	5	32.2222	2.96	0.0123
Date X date X	5	21.0825	1.94	0.0862
Date X date X date X	5	10.4500	0.96	0.4430

Parameter[V]	Class levels[W]	estimate[X]	t for HO:[Y] parameter = 0	pr > |t-value|[Z]
Intercept		4.1343	10.93	0.0001
Ψ	-100 bars	0.0771	0.14	0.8854
	-15 bars	0.8600	1.61	0.1086
	-8 bars	1.0790	2.02	0.0443
	-3.5 bars	0.0029	0.01	0.9957
	-0.1 bar	0.7038	1.32	0.1890
	-0.05 bar	0.0000		
Date		-1.0077	-3.14	0.0018
Date X date		0.1101	1.62	0.1057
Date X date X date		-0.0039	-1.05	0.2937

Table 10. (Continued)

Parameter[v]	Class levels[w]	estimate[x]	t for H_0:[y] parameter = 0	pr>[z] |t-value|
date X Ψ	-100 bars	-0.5860	1.29	0.1971
	-15 bars	-1.1056	2.44	0.0152
	-8 bars	-0.1780	0.39	0.6949
	-3.5 bars	-0.2733	0.60	0.5472
	-0.1 bar	0.4103	-0.90	0.3662
	-0.05 bar	0.0000	.	.
Date X date X Ψ	-100 bars	-0.0672	-0.70	0.4843
	-15 bars	0.1741	-1.81	0.0705
	-8 bars	0.0434	-0.45	0.6515
	-3.5 bars	0.0476	-0.50	0.6201
	-0.1 bar	0.0661	0.69	0.4912
	-0.05 bar	0.0000	.	.
Date X date X date X Ψ	-100 bars	0.0023	0.43	0.6671
	-15 bars	0.0080	1.53	0.1278
	-8 bars	0.0023	0.43	0.6671
	-3.5 bars	0.0023	0.43	0.6671
	-0.1 bar	-0.0031	-0.59	0.5576
	-0.05 bar	0.0000	.	.

[t] Source of variability.
[r] Degrees of freedom.
[s] Probability of exceeding the value of F.
[t] Water potentials at which soil was incubated in these studies.
[u] Sample dates on which soil was assayed for survival of R. solani AG 4.
[v] Parameters included in the regression model
[w] Class levels for which regressions were conducted
[x] Intercept and coefficient estimates for parameters in the model
[y] Students t-test value for the null hypothesis that the parameter equals zero.
[z] Probability of exceeding the absolute value of t.

Saprophytic colonization of rye stem sections by R. solani AG 4 (isolate 1-19) did not occur at -1500 bars (Figs. 18 and 19). However, rye stem pieces were quickly colonized by inoculum added to the soil at all other water potentials tested (Fig. 18). In regression analyses with data from 1 to 12 days after the beginning of the experiment, colonization at -15 bars was shown to be significantly greater than at -0.05 bar; colonization rates at -0.1, -3.5, or -8 bars were not significantly different from that at -0.05 bar (data not shown).

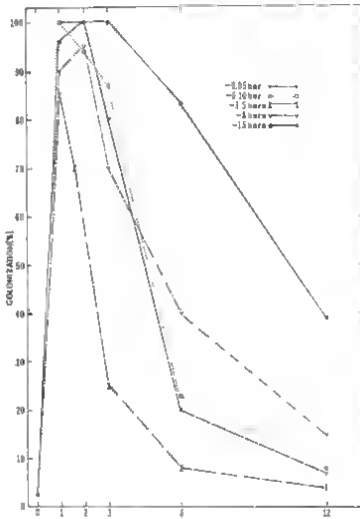

Fig. 18. Saprophytic colonization of rye stem segments by Rhizoctonia solani AG 4 in natural soil held at constant water potentials. Colonization equals percentage of 60 total segments from two experiments colonized by R. solani AG 4.

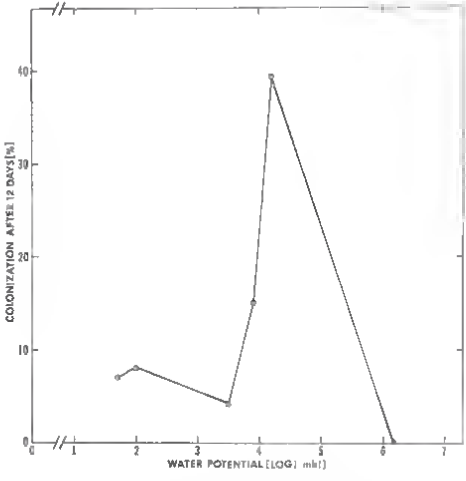

Fig. 19 Saprophytic colonization of rye stem segments by Rhizoctonia solani AG 4 after 12 days incubation in natural soil held of constant water potentials. Colonization equals percentage of 60 total segments from two experiments colonized by R. solani AG 4

SECTION V

DISCUSSION AND CONCLUSION

The anastomosis group concept in R. solani is a meaningful way to divide this variable species (4). Isolates within an AG share common pathological (4), physiological (60, 61), and ecological (4, 58) attributes and have the ability to exchange genetic information (4). Recently workers have used serological data (2), ratios of guaninecytosine (32), and soluble proteins (54) of the different AGs to show that AGs in R. solani are related but distinct species.

My experience with isolates of R. solani and binucleate Rhizoctonia spp. on PDA indicates that isolates from one anastomosis group can be tentatively distinguished from those in another by cultural characteristics alone. Working with isolates of R. solani, others also noted that isolates within an AG usually resemble one another when grown on a given culture medium (28, 58, 65). Although similar culture morphology is not always indicative of this relationship, this recognition becomes useful when one needs to identify large numbers of isolates and absolute accuracy is not necessary. This relationship should not be used, of course, when precise identification of individual isolates is desired.

On the basis of results of tests with Burpee's Bn3I isolate, CAG 3 isolates from the field may represent a subgroup within CAG 3. Burpee et al. (15) did not use Bn3I as a tester isolate in their studies. However, Bn3I anastomosed with 24 of 24 CAG 3 isolates recovered from soil or diseased longleaf pine seedlings from a site 30 km from the experimental field and with an additional

CAG 3 isolate (Rhi5(15)) sent to me by Drs Sumner and Bell, Tifton, GA (data not shown). Subgroups also occur within AG 2 of R. solani (26, 41). Ogoshi (41) divided AG 2 into AG 2-1 and AG 2-2 based on frequency of hyphal anastomosis. Isolates from AG 2-1 do not anastomose with those from AG 2-2; however, bridge isolates exist which are capable of anastomosis with isolates from either subgroup. Although the evidence for a similar phenomenon in CAG 3 is limited, this appears to be the case with isolates of this anastomosis group studied by me.

The existence of binucleate anastomosis groups of Rhizoctonia spp. in the field different from others previously reported (15, 42, 43) is not suprising. Burpee et al. (15) and Ogoshi et al. (42, 43) have identified a total of 17 anastomosis groups among isolates of these fungi; it is likely that others exist (L. L. Burpee, Dept. Environmental Biology, Univ. of Guelph, Ontario N1G 2W1, Canada; personal communication). It is also possible that these isolates are from subgroups of reported anastomosis groups; however, our results provide no evidence for this possibility.

Isolates of AG 4 were commonly recovered from cultivated soil outside the field planted to AG 4 suscepts, but were not recovered from noncultivated soils in the vicinity (all sampled soils were Arredondo fine sands). These results agree with those reported by Kuninaga et al. (33). In studies of noncultivated soils, they recovered isolates from other AGs of R. solani, but not from AG 4. It appears that the presence of AG 4 isolates in the Arredondo fine sands examined in my studies is restricted to areas in which the fungus may function as a parasite.

Soil microbial changes in reduced versus conventional tillage systems have been reported by others (19, 37, 70, 75). In soils planted to winter wheat,

Lynch and Panting (37) described an increase in soil biomass in no-till soils versus tilled soils; they attributed this difference to an increase in fungal biomass. Doran (19) studied surface soils from several different cropping systems, and found consistently higher populations of three groups of microorganisms in no-till soils than in conventionally-tilled soils. In a multicropping study, Sumner et al (70) demonstrated higher population densities of R. solani (predominantly AG 4) and Pythium spp. in surface soil from reduced-tillage systems than from conventionally-tilled systems shortly after planting. Wacha and Tiffany (75) studied a 4-year rotation of corn and soybean. They found no significant quantitative differences between total fungal populations from no-till and conventionally-tilled soils; however, their soil samples were taken at the end of the soybean growing season and after plant debris and thatch had been removed from the soil surface. These factors likely obscured any quantitative differences that may have existed after plowing and during the growing season in this soil.

My results with total fungal population densities agree with those of Lynch and Panting (37), Doran (19), and Sumner et al. (70). When population densities of total fungi were broken into their component genera and species, however, these were not always positively influenced by no-tillage. Although significantly higher densities of Rhizoctonia spp. were often recorded in no-till than in 15-cm till soils, this trend was not found with densities of R. solani AG 4. Rhizoctonia solani AG 4 is a seedling pathogen. Population densities of this species were influenced significantly by the presence of a suceptible host (rye and soybean seedlings, Fig. 9) and the interactions of sample date and soil moisture, sample date and temperature, and soil moisture and temperature

(Table 8), but not by tillage (Table 7). Densities of the pathogen always decreased as time after the seedling stages of its hosts increased. It is apparent that the role of R. solani AG 4 in this field is primarily that of a parasite. Its saprophytic role in the field is probably limited and usually restricted to plant tissues previously colonized when it was a parasite.

Factors other than sample date, soil moisture, and temperature probably influenced the fungi for which regression models were built during the course of this work. Also, for fungi that are sensitive to changes in soil water status (Pythium spp. and P. irregulare) it would have been desirable to assay soil moisture on a more frequent basis than at each sampling date. Any lag in the response of population densities of these fungi to soil water status were not likely detected due to the lengthy intervals between sampling dates. Data from frequent soil moisture assays may have accounted for a higher proportion of the variability calculated for these data than that actually found.

A factor not accounted for in these models that probably contributed a great deal to variability in these data is the spatial distribution of these fungi. Data from studies with Pythium aphanidermatum (64) and R. solani AG 4 and CAG 3(52) have been used to demonstrate a highly aggregated arrangement of propagules of these plant pathogens in soil. A more intensive sampling scheme in the present study would likely have reduced the contribution of this factor to the error term in these data and may have resulted in a greater proportion of the variability in these data being explained by the three factors used in the models.

The results from competitive studies with population densities of Trichoderma spp. (mainly T. harzianum) and Rhizoctonia spp. or R. solani AG 4 are interesting. Trichoderma harzianum is well known hyperparasite of

Rhizoctonia pathogens (6, 40). Although a negative correlation between Trichoderma spp. and these fungi is apparent in plots of raw data (Figs. 20 and 21), densities of Rhizoctonia spp. or R. solani AG 4 were not significantly influenced ($p = 0.05$, data not shown) by densities of Trichoderma spp. when regression analyses were run with transformed (or raw) data. A negative correlation between densities of Trichoderma spp. and total fungi was not evident in plots of raw data (Fig. 22). Data used in the former comparisons may not have accounted for lag periods that would occur before the influence of Trichoderma spp. become apparent in assays for population densities of Rhizoctonia spp. Although such a lag period probably exists, it is not clear how one could account for it with these data. It is possible that data from soil samples taken more frequently than every 5 wk could have provided evidence for these lag periods and could have been used to demonstrate the statistical significance of these trends.

Results from the survival studies conducted for R. solani AG 4 in natural soil at constant water potentials generally agree with those of Dubé (20), and Benson and Baker (9); survival rates in moist soil were lower than those in relatively drier soils. However, results from the present study in air-dried (-1500 bars) and dry (-100 bars) soil apparently contradict those of the above researchers. In their works survival rates, if not enhanced in very dry soil (-940 and -540 bars), were at least more consistent over time than in moister soil. In the present studies, survival of inoculum of R. solani AG 4 was lower in soils held at -100 and -1500 bars than at -15 bars (Figs. 16 and 17); lethal loss of water and an absence of growth activity of the fungus in the drier soil are plausible reasons for this phenomenon. Benson and Baker (9) did not size the

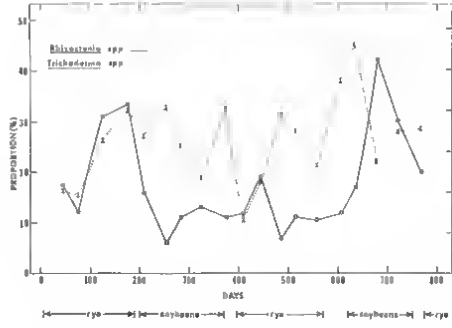

Fig. 20 Relationship between population densities of Rhizoctonia spp. and Trichoderma spp.

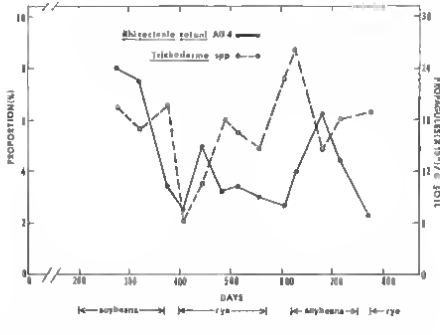

Fig 21. Relationship between population densities of Rhizoctonia solani AG 4 and Trichoderma spp.

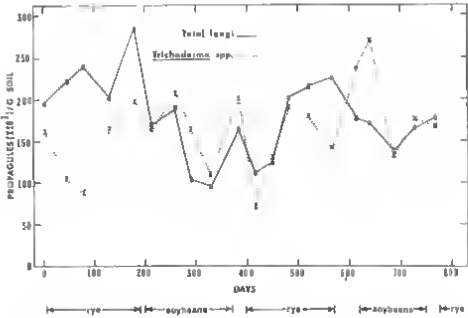

Fig. 22. Relationship between population densities of total fungi and Trichoderma spp.

inoculum used in their studies and it is probable that inoculum used in their work was larger than that used in the present work. Perhaps large inocula would survive better under dry conditions in the soil. Dubé (20) gradually acclimatized his inoculum to drier conditions before use in an experiment. Although it is not known how closely these conditions resembled conditions found in a field situation, this factor may help explain differences between his and the present results.

Survival of R. solani in soil at 0 bar (saturation) has not been previously reported. In the present work it was not possible to distinguish survival rates at 0 and -2 or -10 bars at the 5% level (Table 9). Variability inherent in the systems used in these studies may partially account for these results. It is also possible that statistical analysis at the 5% level may be too stringent when differences in natural soil are being sought (these data were significant at the 12% level, Table 9).

Reduced survival at water potentials of $\leq$ -2 bars or $\leq$ -15 bars in the two series of experiments, is probably partially due to the activity of other microorganisms in the soil. In experiments conducted at -50 bars (data not shown), inocula were quickly colonized by Penicillium citrinum Thom, viability of these inocula were decreased relative to those incubated at -200 bars (data not shown). It is possible that P. citrinum or other microbes were responsible for the reduced survival noted above in moist soils. Rhizoctonia solani AG 4 is probably able to compete with other microbes in these soils when acting as a parasite, but it apparently can not compete well saprophytically.

Although it is unlikely that soil moisture in the experimental field would remain constant for periods of time used in the present survival studies, data

from these studies may still be used to suggest role(s) very moist or very dry soil might play in the survival of R. solani AG 4 in soil under natural conditions. By increasing the activities of other microorganisms or decreasing the availability of O_2, high water potentials ($\geq$ -200 mb) could indirectly effect a reduced survival of this pathogen in field soil. Water potentials in this range could occur during periods of moderate to heavy rainfall or frequent irrigation. Rye or soybean plants would probably be drought-stressed if soil from the surface 5 cm of the field had water potentials of $\leq$ -100 bars. However, during fallow periods or between crops when the soil would not be irrigated, it is possible that water potentials of $\leq$ -100 bars could occur and be responsible for a reduced survival of R. solani in soil as reported by Elmer (23).

From results in the present saprophytic colonization studies, it is clear that, at high initial inoculum densities, R. solani AG 4 is an active saprophyte at a wide range of water potentials. Due to the transitory nature of this activity, however, it is probable that the colonization of rye stem segments in these experiments was superficial. For all water potentials at which colonization occurred, colonization rates decreased rapidly after maximal rates were achieved 1 to 3 days after the start of a study (Fig. 18). It is unlikely that population densities of R. solani AG 4 could substantially increase in the soil used in these experiments through saprophytic activity.

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BIOGRAPHICAL SKETCH

Randy C. Ploetz was born in Gastonia, North Carolina, in 1952. He graduated from Purdue University in 1974 with a B. S. in forestry and in 1976 with a M. S. in plant pathology. From 1977 to 1980 he worked as an assistant in plant pathology at the Bradenton AREC of the University of Florida. In 1980 he began work on his Ph.D at the University of Florida. After graduation he will begin post-doctoral work at the Quincy AREC of the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David J. Mitchell, Chairman
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Raymond N. Gallaher
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Raghaven Charudattan
Professor of Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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